MIMICKING ANHYDROBIOSIS ON SOLID SUPPORTED LIPID BILAYERS

A Thesis

by

VANESSA ALYSS CHAPA

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Chemistry

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Approved by:

Chair of Committee,	Paul S. Cremer
Committee Members,	David H. Russell
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ABSTRACT

Mimicking Anhydrobiosis on Solid Supported

Lipid Bilayers. (May 2006)

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Chair of Advisory Committee: Dr. Paul S. Cremer

The studies presented in this thesis focus on the synthesis of air-stable solid supported lipid bilayers by anhydrobiotic mechanisms. Supported lipid bilayers (SLBs) serve as platforms that mimic cellular membrane surfaces in appearance and behavior. One of the most attractive aspects of the SLB is that it exhibits two-dimensional fluidity that allows for individual components to rearrange as they would in actual cellular membranes. The one thing that would allow the SLB to become an ideal biosensor is the ability to remain stable in the absence of bulk water. As it stands now, unprotected SLBs are unstable in the presence of air causing the membrane to rearrange and delaminate from the surface.

Several biological organisms utilize the process of anhydrobiosis to persevere in severe dehydrated states. Anhydrobiosis occurs when organisms employ large amounts of sugars, particularly disaccharides, to protect their cell membranes. The sugars, often released as a stress response, protect the membrane by replacing the water around the lipid headgroups while also interacting with other sugars to form a glass atop the bilayer.

One of the most successful anhydrobiotic sugars has been trehalose, although other sugars have been evaluated and are capable of protecting lipid bilayers minimally.

The experimental section of this thesis involves the creation of SLBs that are examined with and without the presence of sugar molecules. Essentially, the SLB was created, exposed to sugar solutions, dried, and subsequently rehydrated. Successful experiments occurred when rehydrated bilayers exhibited little damage and were mobile and functional. In addition to trehalose, several other mono- and disaccharides were used as were glycolipids, lipids with sugar headgroups. Upon the completion of all experiments it was clear that trehalose afforded the most protection of all species tested and that glycolipids do not sufficiently protect the membrane during rehydration. Therefore, the addition of a sugar such as trehalose to an SLB could allow for the creation of an air-stable biosensor that would be both practical and require little maintenance.

To Kendall Fruchey

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Thanks goes out to my friends (current and former Aggies) and family who have supported my decisions and who continually make me laugh and smile on a daily basis. Also, thanks to the Cremer group for all their assistance in and outside of the laboratory.

I owe a great debt of gratitude to my mother, father, and brother and would like to thank them for all their love and their constant belief that I can do anything I set my mind to. And finally, to my soon-to-be husband and best friend, thanks for the constant encouragement and willingness to answer any chemistry related inquiry.

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CHAPTER I

INTRODUCTION

Objective

The purpose of my graduate work primarily involved the study of evoking protection on supported lipid bilayers (SLBs) through the use of sugar solutions. Supported lipid bilayers are useful platforms that mimic membrane surfaces and provide a medium for further investigating cellular processes such as ligand-receptor binding and various signaling events.[1-4] Because biomembrane mimics like the SLB make excellent platforms for biosensor devices, it is necessary that upon exposure to air, the system remains unchanged. One novel way in which to create air stable biosensors without permanently altering the bilayer is to mimic the biological process of anhydrobiosis by using a sugar as a protecting agent. In nature, anhydrobiotic organisms such as plant seeds, yeast cells and fungal spores employ large amounts of disaccharides to aid in the preservation process upon desiccation.[5]

The experimental goal of my work was to fabricate a supported bilayer, introduce the sugar of choice, dehydrate and subsequently rehydrate. Along each step of the experimental process, the technique of fluorescence recovery after photobleaching (FRAP) was employed under an inverted microscope in order to evaluate the degree of protection provided by the sugar on the lipid bilayer. Achieving sufficient protection would create a method of drying SLBs that would undergo rehydration to return to their

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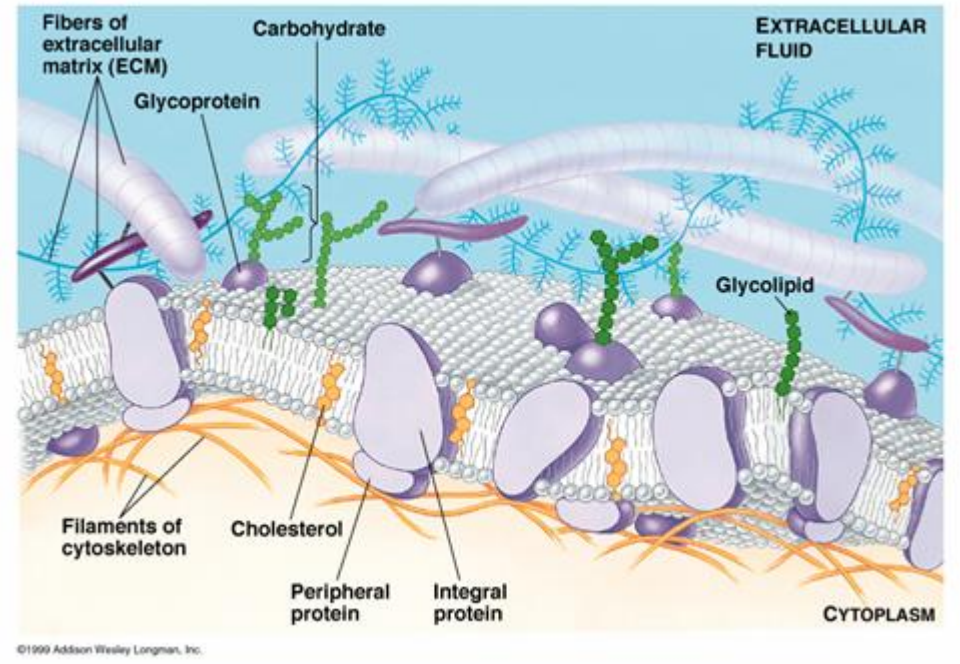

Figure 1.1. Fluid mosaic model.

original state thereby providing a means for the development of air-stable biosensors, sensory devices that would be easy to handle and transport due to their ability to persist once void of water.

Supported Lipid Bilayers

Before focusing on experimental details and results, it is necessary to first become familiar with the medium of choice, the supported lipid bilayer. The supported lipid bilayer is manufactured to mimic the cell membrane.[3, 6] In 1972 Singer and Nicolson proposed the fluid mosaic model (Figure 1.1)[7] which describes the membrane as a fluid mosaic composed of viscous lipids with integral and periphery proteins residing within and atop the bilayer.[8] This two-dimensional model is mimicked via the supported membrane system in order to create a biologically relevant system.

Solid supported lipid bilayers are most commonly formed in one of two ways, vesicle fusion or Langmuir-Blodgett dipping. Vesicle fusion involves the spontaneous adsorption and spreading of unilamellar vesicles upon a substrate (Figure 1.2),[9, 10] while the Langmiur-Blodgett dipping method forms a bilayer by transferring monolayers from an air-water interface to a substrate surface.[11] The more commonly used method of bilayer formation, also the one used experimentally in this study, is vesicle fusion. Upon formation, the new bilayer consists of two rows of phospholipids aligned in such a way that the hydrophobic tails face each other in the center while the hydrophilic headgroups face outwards. A typical bilayer is between 4 and 5 nm thick with bulk water on top and

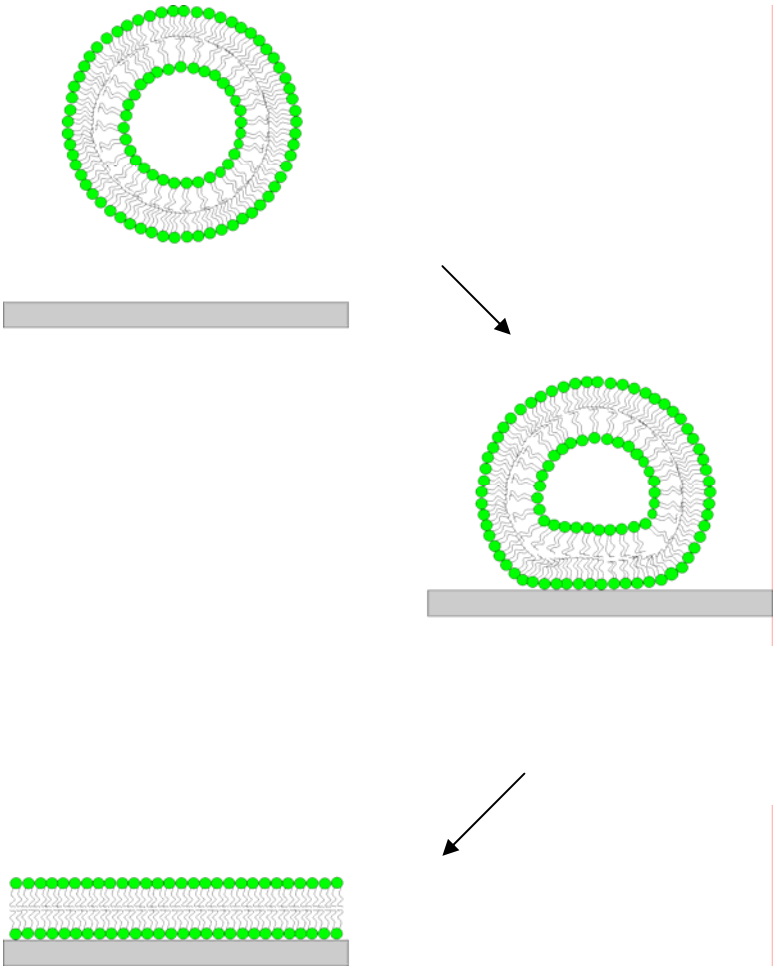

Figure 1.2. Schematic of vesicle fusion.

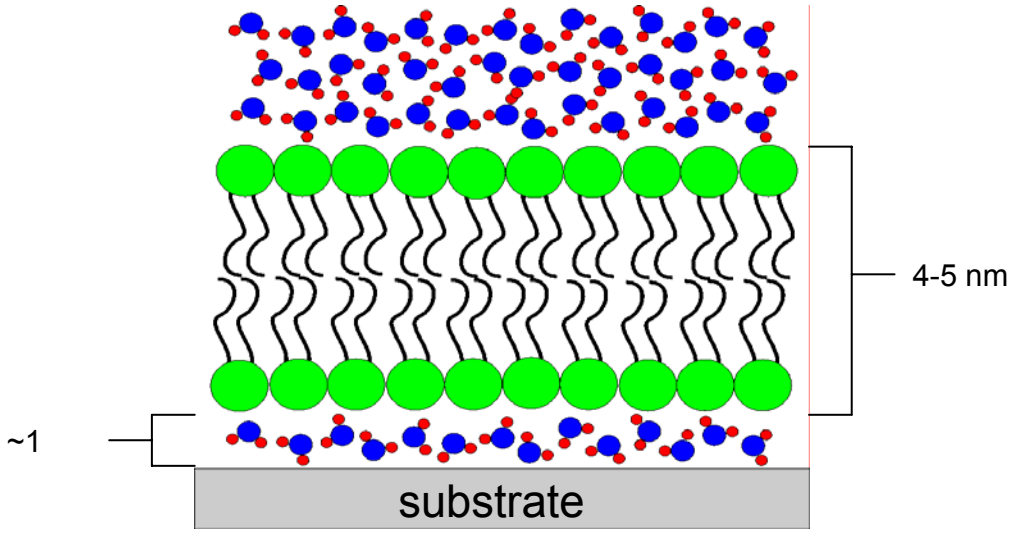

Figure 1.3. Schematic of lipid bilayer. The green circles represent the lipid headgroups with their black tails. The red and blue water molecules reside in a thin layer below the bilayer and in bulk solution atop the layer.

a thin film of water (~1 nm) separating the bottom leaflet of the bilayer and the substrate (Figure 1.3).[12-15] The chemical structure of a lipid phosphatidylcholine, is shown in Figure 1.4 to pictorially display the hydrophobic and hydrophilic regions associated with forming a bilayer.

Of the many advantages associated with SLBs, the high degree of fluidity of the lipids is one of their more valuable assets. Because a supported lipid bilayer exhibits the two-dimensional fluidity[16-19] that allows its individual components to rearrange as in cell membranes, such systems become desirable for biosensing applications. The fluidity of the supported membrane corresponds with the lateral diffusion of the many components within a membrane. Diffusion measurements can be acquired[20] on any SLB and the resulting information can give insight into the biological system that the membrane represents. It is important to note that diffusion within fluid bilayers occurs only in the presence of water and that upon exposure to air, the membrane tends to rearrange and/or delaminate from the surface.[21-28] Delamination occurs when the bilayer is removed from its substrate.

Due to the advantageous properties of supported lipid bilayers, the applications of the mimics are numerous and diverse. With the recent emergence of microfluidic devices, it has become possible to form and specifically pattern supported lipid bilayers on a surface. Such uses of microfluidic devices include the use of laminar flow to form bilayers and then selectively remove certain regions by using a detergent solution.[6] In lieu of using microfluidic devices, a variety of other methods were developed in order to micropattern bilayers on surfaces. Some examples of patterning bilayers include

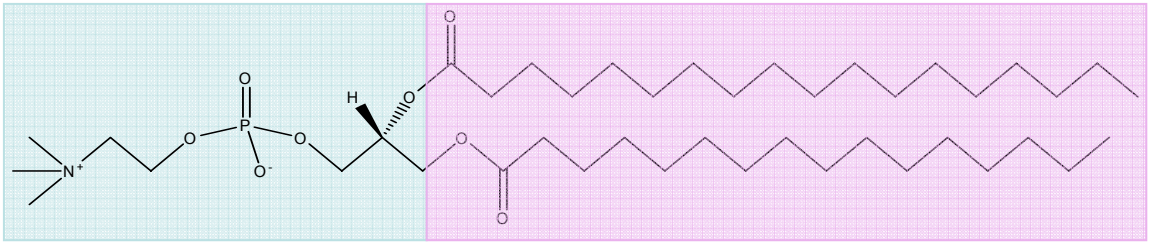

Figure 1.4. Chemical structure of a phosphatidylcholine. The blue region represents the hydrophilic headgroup while the pink areas highlights the hydrophobic tails of the lipid.

the creation of barriers in bilayers through scratches in the substrate[16] and the use of hydrophobic boxes on a surface to create spatially addressed arrays of lipids.[4] Another method used to pattern bilayers involves the selective polymerization of a biomimetic membrane that contains photopolymerizable lipids.[26] All lipids not exposed to UV irradiation can be rinsed away leaving a pattered array of lipid bilayers on the surface.

Due to their resemblance to cell membranes, supported lipid bilayers are excellent platforms for the study of many biological processes. One area of interest in terms of the biological applications of SLBs is the study of ligand-receptor binding. In particular, several groups have studied antibody binding in a bilayer as an effect of hapten, a small molecule which elicits an immune response.[20, 29] While SLBs have many characteristics in common with an actual cell membrane, one aspect that is difficult to reproduce is the presence of proteins within the bilayer. This task is often difficult because while researchers have been able to reconstitute integral membrane proteins within bilayers using linking agents,[30] it has proven difficult to insert proteins without using a linker or a cushion. Proteins cannot be inserted into a membrane without additives due to the strong interactions that proteins experience with the solid support.

Previous Studies on Air-Stable SLBs

While it has been demonstrated that fully hydrated supported lipid bilayers serve as excellent platforms for sensor devices, the fact remains that upon exposure to the air/water interface, the bilayer experiences severe damage. Consequently, air stability has been investigated on SLBs by employing various methods of either chemical

modification or incorporation of stabilizing agents within or on top of the bilayer surface. Unlike the methods described below, the goals of my graduate work are unique in that by protecting bilayers with sugars, no modification of the bilayer is necessary. Essentially, an unaltered, single-component lipid bilayer can maintain its function after severe dehydration with the addition of certain sugars.

Earlier attempts of air-stable supported lipid bilayers involved the incorporation of stabilizing agents within the bilayer itself. One strategy utilizes the lipid bis-sorbylphosphatidylcholine which upon polymerization forms a cross-linked structure.[23] While the polymerization takes place under water, once cross-linking has occurred, the bilayer can be exposed to air. Once rehydrated, the cross-linked lipids retain the same protein resistance that was present before water removal. Yet another experiment demonstrated that positively charged lipids within a bilayer creates a stable electrostatic interaction with a poly(dimethylsiloxane) surface.[28, 31] The positive charge of the lipids aided in creating an air-stable bilayer that retained membrane fluidity once the system was rehydrated. In fact, lateral diffusion coefficients were virtually unchanged upon rehydration whereas control experiments with uncharged lipids showed a nominal decrease in diffusion after air exposure.

Other attempts at creating air-stable SLBs relied on incorporating moieties on top of the bilayer rather than within the membrane mimic. One such attempt involved the use of a protective protein layer composed of streptavidin that was successful in pinning down the ends of a bilayer thus prohibiting disruption of lipid ordering.[32] The monolayer of protein stiffened the bilayer allowing for water to be removed without

delamination of the bilayer. In this experiment, the components of the bilayer were not modified. More recent attempts at bilayer stabilization involved the addition of pegylated lipopolymers that protect the bilayer in a size/polymer conformation dependent manner.[33] The addition of poly(ethylene glycol) to lipid headgroups stiffened the bilayer preventing delamination once water was removed. In this experiment, it was noted that protection was dependent upon PEG density and that whenever protection was high, bilayer fluidity remained intact.

While it has been shown that modification of lipid bilayers can aid in the creation of air-stable SLBs, it has become desirable to create a system in which no modification is necessary. With this in mind, my graduate work focused on protecting an unmodified bilayer with sugar molecules that would protect the system after it was exposed to air, but that could be removed upon rehydration. With the success of such a system, it is plausible to create and protect biosensors without having to perform any alterations to the original bilayer composition.

CHAPTER II

ANHYDROBIOSIS

Overview

While water is often a necessity for biological survival, anhydrobiotic organisms have proven that life can persist in a dehydrated state. The key to survival in dehydrated states is to maintain the cell's integrity, specifically its membrane. The membrane, a primary target of cellular destruction, loses diffusion, and therefore all modes of transport within and among cells when in a severely dehydrated state. Upon desiccation, anhydrobiotic organisms utilize large quantities of disaccharides to aid in the preservation process.[5] While many disaccharides provide sufficient protection in dry environments, trehalose has proven to be one of the most effective membrane preservation agents with applications in the pharmaceutical, medical and food industries.[34]

Long before sugars, in particular disaccharides, were used in laboratory settings as protectors during dehydration, they were already being utilized by anhydrobiotic organisms. It has been shown that anhydrobiotic organisms, when compared to non-anhydrobiotic species, have higher concentrations of disaccharides, especially trehalose, within them.[5, 35-38] Such is the case with the nematode *Aphelenchus avenae*[39] (Figure 2.1) which upon slow dehydration coverts up to 20% of its dry weight into trehalose.[40] The extent of survival of organisms such as *Aphelenchus avenae* and bakers yeast (*Saccharomyces cerevisiae*) is directly dependent on the synthesis and accumulation of trehalose.[41-43] In some organisms such as archaebacteria, the synthesis of trehalose

Figure 2.1. Picture of female *Aphelenchus avenae.* The head is to the right of the image and the brown coloration is due to the immunoreaction of LEA protein.

occurs in response to stress created by the onset of dry conditions.[44]

While it has been well established that certain organisms practice anhydrobiosis in order to thrive even in the driest of conditions, researchers began to study laboratory synthesized systems in order to gain a deeper understanding of the preservation process. With an understanding of the chemistry of sugars and the nature of cell surfaces, scientists could propose mechanisms by which cell membranes are protected. With the addition of experiments using liposomes and vesicles as cell mimics, more understanding about the nature of these protecting sugars is gained. More recently, experiments have been performed on supported lipid bilayers, cellular mimics that are important in the growing field of biosensor design and application.

Chemistry of Saccharides

Before one can fully understand the mechanisms of anhydrobiosis, it is necessary to have a basic knowledge of the chemistry of the sugars that are involved. The simplest sugars are monosaccharides, compounds that have a single polyhydroxy aldehyde or ketone unit.[45] The most abundant aldose and monosaccharide is D-glucose while the most common ketose is D-fructose. As displayed in the Fischer projections in Figure 2.2, the difference between an aldose and a ketose is the position of the molecules carbonyl group. The size of a monosaccharide depends of the length of its carbon backbone, which can range in length between two to seven carbon atoms.

While it is simpler to show monosaccharides as straight-chained molecules, once in aqueous solution, monosaccharides with 5 or more carbons exist as cyclic molecules.

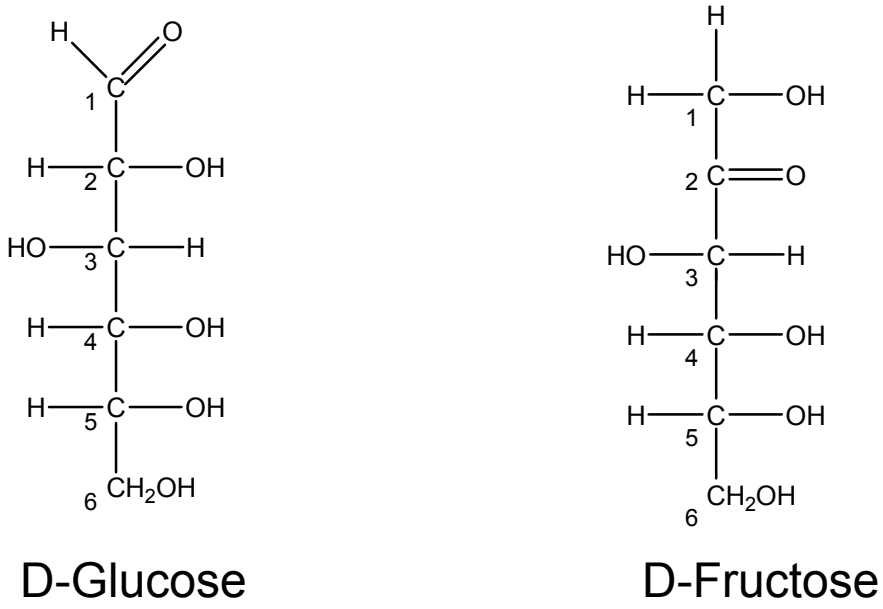

Figure 2.2. Fischer projections of glucose and fructose. The carbon backbone is numbered to distinguish the difference in carbonyl position.

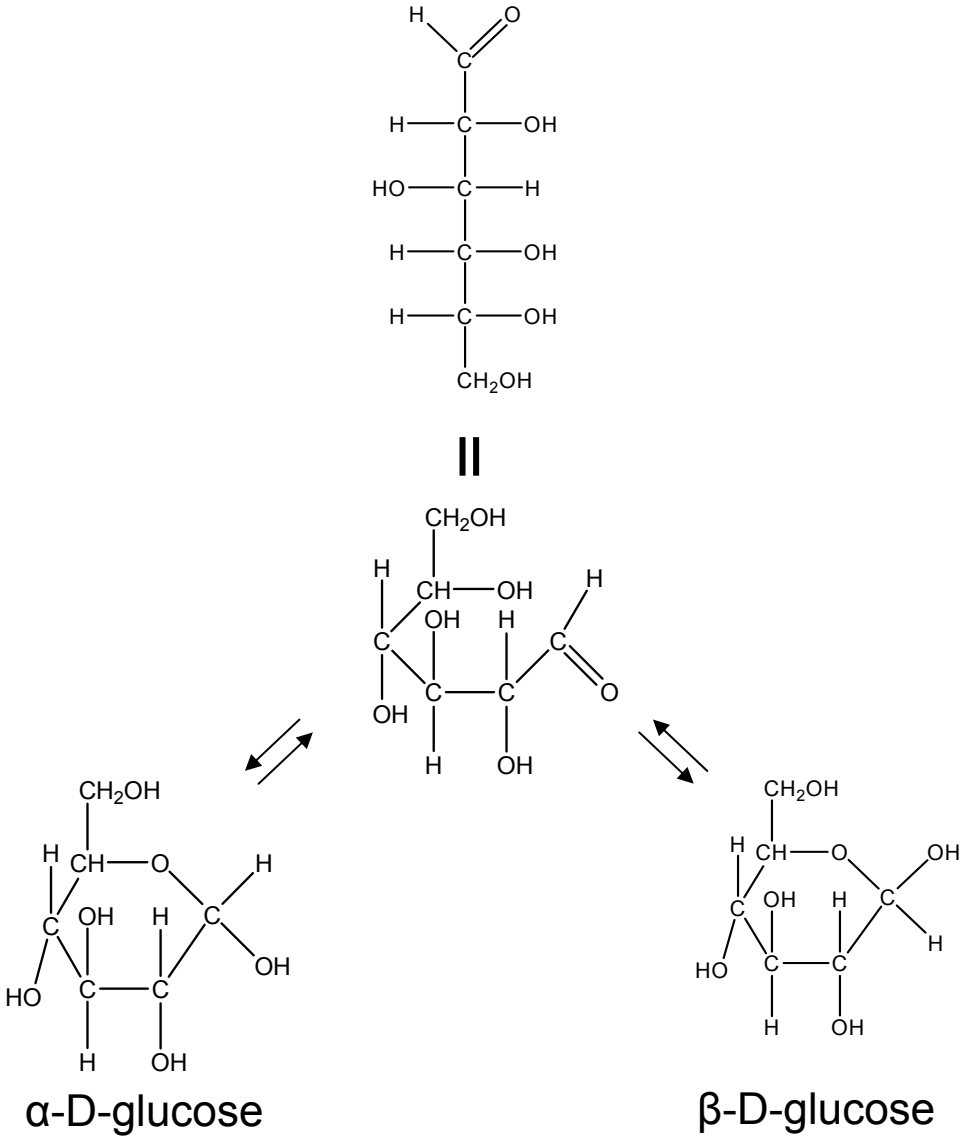

Figure 2.3. Cyclic formation of α and β-D-glucose.

Cyclic rings form when an aldehyde or ketone group reacts with an alcohol group to form sugar derivatives entitled hemiacetals or hemiketals. The primary carbon of the reaction, the carbonyl carbon of the aldehyde or ketone, is termed the anomeric carbon and based on the positioning around this moiety, two stereoisomers can exist. For examples, the cyclization of linear glucose results in the formation of the two anomers α-D-glucose and β-D-glucose (Figure 2.3). In aqueous solution, the two anomers interconvert through the process of mutarotation thereby creating an equilibrium mixture.

The glycosidic bond within a monosaccharide is the bond that connects the anomeric carbon to the acetal oxygen.[46] Disaccharides are formed when there is linkage between neighboring monosaccharide units via a glycosidic bond. While trehalose has well been established as the sugar with the greatest protection capabilities in anhydrobiotic organisms, my graduate work investigated the efficiency of other disaccharides as well. Sucrose, the most abundant naturally occurring disaccharide, consists of a glucose and a fructose unit and was experimentally tested for its degree of bilayer protection. Trehalose is formed when two glucose units combine into a disaccharide by forming an α(1→1) glycosidic bond between the glucose molecules.[45] On the other hand, maltose is formed when there is an α(1→4) linkage between glucose subunits. The difference in where the glycosidic bond occurs in trehalose and maltose is demonstrated in Figure 2.4, as is the presence of a reducing end in maltose but not in trehalose. In disaccharides and polysaccharides, a reducing end exists when the end of

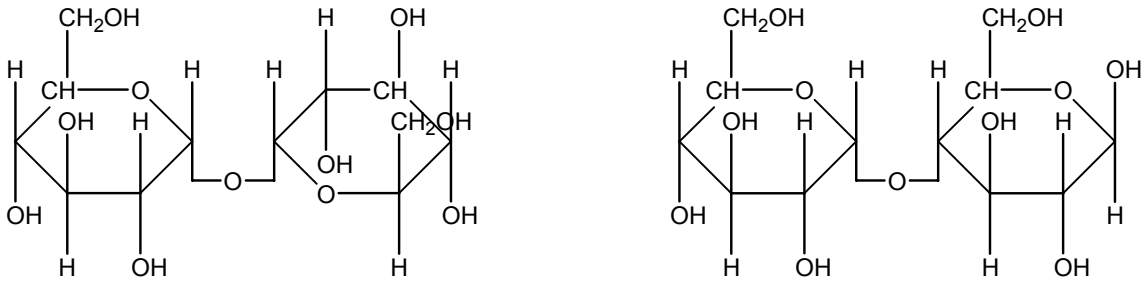

Figure 2.4. Chemical structures of trehalose and maltose.

the chain of subunits has a free anomeric carbon. Reducing sugars can act as reducing agents in reactions such as the Maillard reaction, a non-enzymatic browning reaction.

As will be shown later, trehalose and maltose give very different results in terms of protecting SLBs and it will be proposed that this occurs due to their differing 3-D structures which correspond to the differences in where the glucose subunits are bound. Additionally, other mono- and disaccharides will be examined for their effectiveness in providing protection under anhydrobiotic conditions.

Mechanisms of Anhydrobiosis

While the existence of anhydrobiotic organisms is unquestioned, the mechanisms by which anhydrobiosis occurs is still a matter of extensive study. Through careful study of the lipid bilayers that constitute the cellular membrane, it was possible to determine how dehydration damaged the membrane. Mechanisms by which sugars preserve membranes have been widely discussed. One of the first proposed mechanisms hypothesized that water molecules near membrane surfaces are replaced with sugar via hydrogen bonding.[47] More recent hypotheses, however, suggest that it is not only the presence of the sugar, but the interactions among sugar molecules to form a glass-like coating, that preserves the biomembrane during anhydrobiosis.

The two primary stresses that affect bilayers during dehydration are fusion and lipid phase transitions.[5, 35-37] Early examination of fusion between vesicles by resonance energy transfer of fluorescent probes or laser light scattering indicated that sugars such as trehalose inhibit fusion during drying.[43, 48] When unprotected lipid bilayers are dried,

headgroup packing increases and there is in increase in van der Waals' interactions of the carbon tails thus resulting in a phase transition temperature (T_m) increase.[5] With an increase in T_m, dry bilayers without trehalose tend to leak once rehydration is initiated. When trehalose is present, however, the T_m is depressed to such an extent that leakage is avoided and the bilayer maintains its liquid crystalline phase (Figure 2.5) even without water.[48, 49]

In 1973, the water replacement theory was proposed hypothesizing that sugars replace the water around the polar moieties of membranes and proteins thereby providing stabilization in dry states.[5, 47] The disaccharide trehalose, a clear frontrunner in terms of bilayer stability, is said to achieve this through the direct interaction between its –OH groups and the phosphates of the lipids.[36, 37, 50-52] In fact, modeling based on X-ray coordinates suggests that given its stereochemistry, trehalose packs tightly between lipid polar head groups providing ample opportunity for interactions.[53-56] Such models strongly suggest that in order to accommodate the trehalose, van der Waals' interactions between hydrocarbon chains must be decreased and consequently, so must the T_m. Later models, however, suggested that the stereochemistry of trehalose may not be the sole factor incurring stabilization during rehydration and that other avenues may be involved.[54]

In the last ten years, an alternative mechanism of anhydrobiosis, the formation of sugar glasses, or vitrification, has been proposed to work in conjunction with water replacement to stabilize dry bilayers.[57] Glasses are homogeneous phases that have a

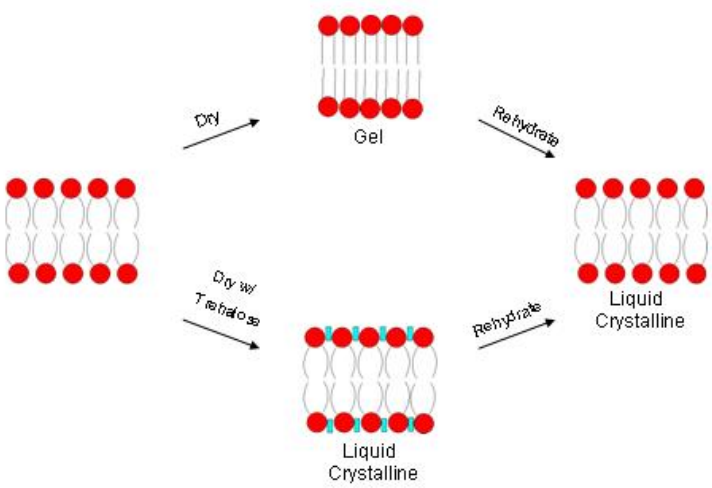

Figure 2.5. Diagram illustrating the mechanism of trehalose stabilization during anhydrobiosis. The blue rectangles represent the trehalose molecules that preserve the liquid crystalline phase of the bilayer.

temperature-dependent transition from the solid to a more viscous material at the glass transition temperature (T_g).[58] When a sugar glass forms atop a bilayer under dryconditions, the phase transition temperature (T_m) of the bilayer decreases due to increased headgroup packing and van der Waals' interactions.[47] Due to a lower T_m, the lipids will undergo a transition from dried to rehydrated form with minimal damage. The first experimental proof of sugar glasses was performed in 1989, when differential scanning calorimetry (DSC) was used to detect clear glass transitions of corn embryos taken from dry seeds.[59] As shown in Figure 2.5, a bilayer dried in the presence of a sugar such as trehalose remains liquid crystalline when dehydrated and although the specifics remain unclear, it has been proposed that vitrification inhibits the increase in T_m.[60]

Previous Studies of Liposomes

Whereas the use of supported lipid bilayers in anhydrobiotic studies is novel, many experiments on liposomes have been performed frequently with varying methodologies. Studies of the effects of sugars on liposomes were performed by Fourier transform infrared spectroscopy,[61-64] differential scanning calorimetry,[49, 65] molecular dynamics simulations,[66-71] solid-state NMR,[72] electrochemical methods,[73, 74] and osmotic measurements.[75, 76] Other experiments performed used very large sugars such as fructans,[77, 78] and liposomes containing cholates[79] and surfactants.[80]

Fourier transform infrared (FTIR) spectroscopy has become an important

investigative tool in the determination of the interactions that occur between sugars and the lipids of biological membranes. FTIR was used to study two-component liposomes dried in the absence and presence of trehalose.[62] Resulting infrared spectra indicated that the trehalose had a fluidizing effect on one of the components that strengthened when heat was applied to the dried liposomes, indicating that sugar/head group interaction became enhanced with heat addition. A later study used a variety of sugars including sucrose, glucose, raffinose, trehalose, maltose and dextran to study hydrogen-bonding as an affect of sugar size.[64] Band positions of the OH stretch mode demonstrated a correlation between sugar size and the extent of interaction with the vesicles. Accompanying DSC studies were also able to relate FTIR spectrum with the T_g for each sugar used.

Differential scanning calorimetry (DSC) is a useful device in which to determine the phase transition temperatures of sugars utilized as bilayer protectants. Earlier work examined dry mixtures of DPPC lipids with and without trehalose present.[49] Results confirmed the affect of trehalose on the phase transition temperature (T_m) as the minimum T_m of the system with trehalose was 24°C while the vesicles without sugar had a transition between 105 and 112°C. DSC experiments were also used to study the effects of highly concentrated branched oligosaccharides (HBOS) on the protein bovine serum albumin (BSA).[65] With a T_m of -16.1°C, higher than that of sucrose, it was determined that the protein was preserved as trapped water and HBOS caused the structure to stiffen and become rigid.

A simulated method of understanding the effects of sugars on membranes involves the use of computerized molecular dynamic studies. Molecular dynamic studies are numerous and convenient in that depending on how the system is constructed, factors from water structure to headgroup angles can be analyzed. While much of the earlier laboratory experimentation dealt more with liposomes, the flexibility of molecular simulations allows for systems that deal with the planar lipid bilayer. One such study performed molecular simulations of DPPC lipid bilayers with and without trehalose and sucrose.[66] Simulated areas per headgroup of the lipids were calculated before and after the addition of disaccharides and it was noted that areas did not change when sugar interactions were present. Upon inspection of all computations, it was determined that both disaccharides interacted only at the bilayer surface by forming multiple hydrogen bonds to the lipid, thereby supporting previous experiments.

While the basis for most of the anhydrobiotic simulations on bilayers is consistent from experiment to experiment, variations of bilayer compositions as well as changing other factors enable researchers to obtain numerous results. Simulations performed on a bilayer system containing cholesterol were performed with trehalose as the disaccharide.[81] Cholesterol resides within the hydrophobic tails of the lipids and changes lipid characteristics such as packing, diffusion and permeability. Trehalose effectively protects the bilayer even when cholesterol is present in the tail regions and changes in molecular dynamics indicated that trehalose and cholesterol affect the bilayer dynamics in very different ways.

While much of the earlier anhydrobiotic experiments consistently examine trehalose and other disaccharides, some research has involved less studied sugars and cell membrane systems. The study of fructans, polysaccharides consisting of one glucose unit and two or more fructose units, was performed to determine the effect of polysaccharides on membranes.[78] Due to its strong hydrophobic nature, fructans tended to have a stronger effect on the lipids than other polysaccharide counterparts. This experimental proof led to the proposal that fructans not only store carbohydrates in plants, but they may also protect plant species in cold, dry conditions. Another recent experiment performed analysis on cholate, the salt of cholic acid, containing liposomes that were surrounded by "matrixes" of trehalose or sucrose.[79] The reason for the addition of the cholate was to create a mimic of a transferosomes, liposomes that contain lipids and sodium cholate and are more hydrophilic and flexible. Conclusions from this study indicate that the presence of the cholate lead to different water interactions within the lipids which thereby alters sugar-lipid interactions while giving the liposome improved flexibility.

Previous Studies of SLBs

While all previous experimentation studied the affects of sugars on lipid membranes, none had utilized the solid supported lipid bilayer as a means for investigation. While completing my studies of anhydrobiosis on SLBs via microscopy, an investigation was completed in which the cellular mimics were visually inspected via atomic force microscopy (AFM).[82]

In this series of experiments, AFM was used to examine SLBs composed of sphingomyelin, dioleoylphosphatidylcholine, and cholesterol when they were dried and rehydrated in the presence of stabilizing components including trehalose, glucose, sucrose, dimethyl sulfoxide (DMSO) and Dextran T70. Multi-component membranes were used in order to mimic biological membranes that undergo lipidic domain formation which can be seen by the topographical images obtained by the AFM. Probing by AFM also gave visualization to any damage experienced during the dehydration process. Upon visual inspection, it became clear that trehalose and sucrose provided good protection while DMSO, glucose and dextran provided insufficient protection when in the dehydrated state. Microdomains did not fuse or enlarge when adequately protected by trehalose and sucrose further supporting the fact that good protecting sugars prevent damage to the biological functioning of cells.

Clearly the experimentation on anhydrobiosis has been numerous and extensive, but has mainly been focused on vesicle and liposome systems. The interest in studying anhydrobiosis on SLBs is valid because it provides the opportunity to study biological processes of a platform that is convenient and easy to work with. More importantly, if one can obtain ample protection on an SLB by sugars such as trehalose, it becomes possible to create an air-stable platform. In my attempts, I hoped to create an avenue by which one can create a biosensor that can be protected with sugar and dehydrated allowing for a return to its original state upon rehydration.

CHAPTER III

MIMICKING ANHYDROBIOSIS ON SUPPORTED LIPID MEMBRANES: A COMPARISON OF FREE SUGARS AND GLYCOLIPIDS

Synopsis

A series of experiments using sugars, including trehalose, was performed to test the protection on supported lipid bilayers upon air exposure. Additionally, several glycolipids were incorporated into bilayer systems to see if protection in dehydrated states could be achieved. Fluorescence recovery after photobleaching experiments were performed on bilayer samples in order to compare conditions before and after dehydration. Results indicated that trehalose was the most effective sugar for protecting bilayers exposed to air and allowed nearly full recovery of bilayer fluidity after return to the hydrated state. Maltose, a reductive disaccharide, protected bilayers from delamination, yet did not afford fluidity to membranes upon rehydration. Moreover, glycolipids offered little to no protection against bilayer delamination upon subsequent rehydration. These results indicate that only sugars which fulfill certain size, geometric and chemical criteria can be employed to protect supported lipid bilayers from destruction upon air exposure thereby creating biosensors that can be protected without any modification.

Introduction

The biological process of anhydrobiosis occurs in certain organisms as a means of survival in dry states.[5] When said organisms begin to experience the stresses of

dehydration, their automatic response is to produce large amounts of sugars, in particular, disaccharides. While many disaccharides provide some protection to the organism and its cell membrane, trehalose has proven to be one of the most effective agents in providing protection.[34] Through the replacement of water and vitrification, sugar molecules protect the cell membrane allowing for the return of cellular function once rehydration has occurred. Although anhydrobiosis has been studied on liposome and vesicle solutions, the emergence of supported lipid bilayers as biosensor devices has led to new investigations of anhydrobiosis on planar lipid bilayers. Mimicking anhydrobiosis on an SLB could afford the development of simple, air-stable biosensors.

Unlike other works of anhydrobiotic study, my work focused on studying the process on supported lipid bilayers (SLBs). Supported bilayer systems provide cellular mimics while also serving as excellent biosensing platforms. Through the use of fluorescence microscopy, in particular fluorescence recovery after photobleaching (FRAP), it became possible to analyze sugars and their effectiveness in protecting SLBs. While the more common anhydrobiotic sugars such as trehalose, sucrose and glucose were tested for their ability to protect in dry conditions, it became desirous to study other sugars that have had limited research focus. In addition to trehalose, sucrose, and glucose, lactose and maltose were added to the list of so-called "free" sugars.

Upon completion of preliminary experiments that showed that trehalose and other sugars, to some extent, did indeed protect the SLB, it was decided that glycolipids would be novel sugars to examine. While glycolipids, lipid moieties that have sugar headgroups, are biologically important as components of the cellular glycocalix,

questions arose during this study as to whether or not they could protect the bilayer as well as sugars that are free in solution. Experiments involving several glycoplipids were utilized in order to answer such questions.

Herein we compare the efficacy of several simple carbohydrates and glycolipids on protecting supported phospholipid bilayers upon air exposure. Evaluation of the membranes is performed by fluorescence microscopy and fluorescence recovery after photobleaching (FRAP). The data clearly indicates that glycolipids are relatively ineffective at protecting lipid bilayers against delamination from the surface and/or extensive damage. Adding simple sugars to the buffer solution before air exposure proved to be more effective in some cases. Notably, trehalose provided excellent protection against loss of membrane fluidity and delamination. On the other hand, maltose, which has the same molecular weight as trehalose, prevented delamination but did not preserve membrane fluidity upon rehydration. Glucose, sucrose, and lactose provided even less protection than maltose.

Experimental

Materials. 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), G_{M1} ganglioside, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-lactosyl (Lactosyl PE), and glucosylcerebroside were purchased from Avanti Polar Lipids (Alabaster, AL). *N*-(Texas Red sulfonyl)-1,2-dihexadecanoyl-*sn*-glycero-3-phophoethanolamine (Texas Red DHPE) was obtained from Molecular Probes (Eugene, OR). D(+)-Glucose, D(+)-maltose monohydrate, and D(+)-sucrose were purchased from Acros Organics (Morris

Plains, NJ). D(+)-Trehalose was purchased from Fluka BioChemika (Buchs, CH) and β-lactose was purchased from Sigma-Aldrich (St. Louis, MO). Purified water with a minimum resistivity of 18.2 MΩ·cm was obtained from a NANOpure ultrapure water system (Barnstead, Dubuque, IA). Phosphate-buffered saline (PBS) solution was prepared with 10.0 mM sodium phosphate and 150 mM NaCl (Sigma-Aldrich). The pH of the buffer was adjusted to 7.4 by adding NaOH (EM Science). Poly(dimethylsiloxane) (PDMS) was used to prepare PDMS wells. Glass microscope slides (VWR International) were cleaned and annealed by a previously determined procedure.[83]

Preparation of Unilamellar Vesicles and Bilayer Formation. Small unilamellar vesicles (SUVs)[84] were typically prepared with POPC and 0.1 mol % Texas Red DHPE as a fluorescent probe. Vesicles for the glycolipid studies were prepared with an appropriate mole percent of either G_{M1}, Lactosyl PE, or Glucosylcerebroside in addition to POPC and the dye-conjugated lipid. For all studies, solutions with appropriate concentrations of each lipid component were mixed in chloroform. A stream of dry nitrogen was used to evaporate the solvent and the remaining dried lipids were desiccated under vacuum for at least 3 hours. The lipids were then rehydrated in PBS to a final concentration of 2.5 mg/ml and were subject to 10 freeze-thaw cycles. The resulting vesicle solutions were extruded through a polycarbonate filter (VWR International) with an average pore size of 50 nm Dynamic light scattering by a 90Plus particle size analyzer (Brookhaven Instruments Corp.) showed that resulting vesicles had

diameters of approximately 90 nm, but were slightly dependent on the specific chemistry of the liposome.

Supported lipid bilayers were formed via vesicle fusion inside a PDMS well adhered to a clean glass coverslip. After an incubation period of 10 minutes, the well was rinsed thoroughly with deionized water. The sample was then observed with the 10x objective of an inverted epifluorescence Nikon Eclipse TE2000-U microscope. Images were taken with a MicroMax 1024b CCD camera (Princeton Instruments) and subsequent data analysis was performed with MetaMorph software (Universal Imaging).

Fluorescence Recovery after Photobleaching (FRAP). In order to quantitatively measure the motions of molecules, in this case lipids within a bilayer, it is necessary to employ the fluorescence technique of FRAP. This technique can be used if a fluid bilayer contains a small percentage of fluorescently labeled lipids. By subjecting a small area of the bilayer to a strong, focused laser beam, the area becomes photobleached and the resulting fluorescence image shows a dark spot where the bleaching has occurred. As soon as the lipids have been photobleached, neighboring unbleached lipids begin to move into the dark region at a specific diffusion rate. If the spot remains dark, the lipid bilayer is labeled immobile. It is possible to also determine the percent recovery of a bilayer by comparing the fluorescence detected after all diffusion has occurred to the fluorescence of the bilayer before photobleaching.

In this study FRAP measurements were performed by focusing laser light from a 2.5 W Ar^+/Kr^+ laser (Stabilite 2018, Spectra Physics) onto the planar bilayer. Radiation at 568.2 nm was exposed to the surface for periods of ~1 sec at 100 mW. By focusing

the laser onto the bilayer through the 10x objective, a 17.7 μm full width at half-maximum bleach spot was produced. Recovery of the bleach spot was recorded by time-lapse imaging. The fluorescence intensity of the bleached spot was taken as a function of time after background subtraction and normalization of intensity. The recovery of fluorescence intensity inside the bleached spot was monitored as a function of time and fit to single exponentials. From these curves, mobile fraction of the dye-label lipids and the half-time of recovery, $t_{1/2}$, were determined via previously established procecdures.[85]

Dehydration and Rehydration of Lipid Bilayers. For the studies with aqueous sugars, freshly formed bilayers were visually examined by fluorescence microscopy and also analyzed with FRAP measurements first in the absence of the sugar. Sugar solutions of trehalose, maltose, sucrose, glucose or lactose were prepared in PBS solution where the amount of sugar in solution is given as a weight percent (w/w %). The water above the bilayer was exchanged for the desired sugar solution and this was allowed to incubate for approximately 1 hour. Following the incubation period, the lipid bilayer was once again examined via microscopy and FRAP to verify that no major changes had occurred. To dehydrate the bilayer, the bulk sugar solution was removed from the PDMS well with a pipette and the platform was placed under vacuum in a desiccator for 1 hour followed by exposure to ambient air for another 20 hours. After desiccation, the dried bilayer was again observed again by microscope to visually inspect for delamination. Deionized water was placed in the well and the system was allowed to rehydrate for 1 hour at which time thorough rinsing was performed. Epifluorescence images and FRAP data were taken of the rehydrated bilayer. For the series of

experiments involving glycolipids, procedures were performed in a similar manner to the previously described method. Although in this case no sugar solution was introduced above the bilayer before air exposure.

In all experiments, delaminated areas were qualitatively assayed with MetaMorph software by dividing the area of non-uniform fluorescence regions by the total area to obtain an approximate percent damage. Percent delamination values were obtained by averaging the delaminations observed in twelve regions of three samples per each sugar solution. Since only a portion of each sample was visually inspected for delamination, the average percent delamination is purely a qualitative assessment and is used to gauge the effectiveness of all the sugars when compared to each other. To help measure background fluorescence, the bilayer coated substrates were scratched with the tip of a pair of sharp tweezers.

Results

Protection of Supported Lipid Bilayers by Sugars. Trehalose, maltose, glucose, sucrose and lactose, were each used as candidate bilayer protectants. There structures are provided in Figure 3.1. Sugar solutions were prepared and tested at 15 and 20 w/w % for each of the five sugars. In the case of trehalose, sugar solutions of 5 and 10 w/w % were also employed. FRAP data were obtained for bilayers before and after dehydration, and images were taken before sugar addition, after drying the bilayer, and when the system was rehydrated.

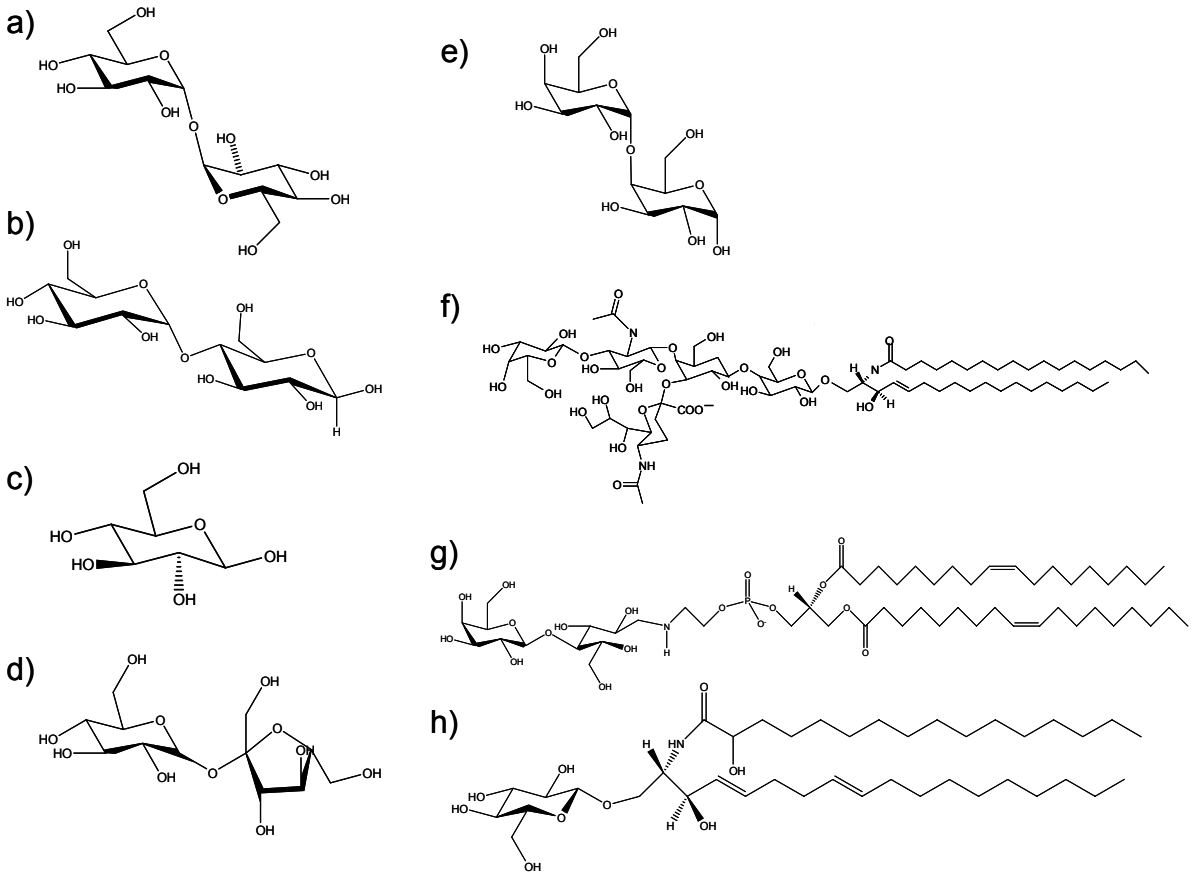

Figure 3.1. Structures of sugars used in experiments. Free sugars: a) trehalose, b) maltose, c) glucose, d) sucrose, and e) lactose. Lipids with attached sugars: e) ganglioside G_{M1}, f) 18:1 lactosyl PE, and g) glucosylceramide.

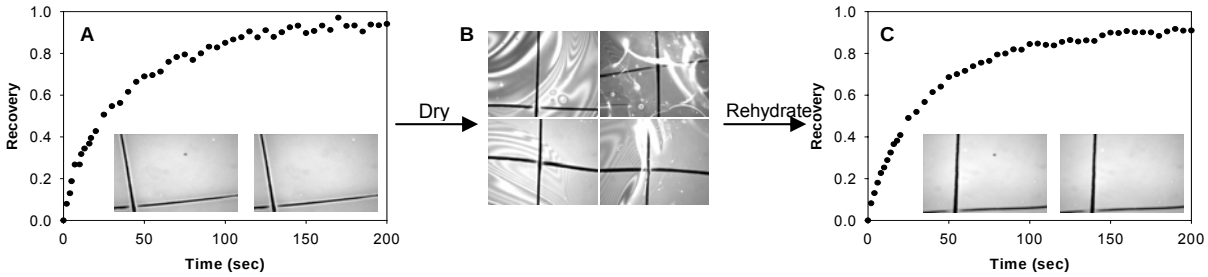

Figure 3.2. FRAP curves from POPC bilayers on planar borosilicate substrates. A) recovery curve for bilayer with no trehalose present; B) images of bilayer with trehalose that has been dried; C) recovery curve for bilayer that has been rehydrated after treatment with trehalose.

Supported phospholipids bilayers composed of 99.9 mol % POPC and 0.1 mol % Texas Red DHPE were first characterized in PBS at pH 7.4 with 150 mM NaCl to insure proper supported bilayer formation. FRAP data of the uniform bilayers was obtained and the diffusion constant D was determined by plugging the measured value of $\tau_{1/2}$ into the following equation:

$$D = w^2 / 4 \tau_{1/2} \gamma_D$$

where w is the full width at half-maximum of the Gaussian profile of the focused beam and γ_D is a correction factor that depends on the bleach time and the geometry of the laser beam.[85] For all FRAP experiments in this paper, w = 17.7 μm and γ_D = 1.2. Diffusion constants and percent recoveries were also obtained on bilayers once desiccation and rehydration were performed.

The recovery curve and inset pictures in Figure 3.2A are for a POPC/Texas Red DHPE bilayer. These experiments were repeated after the introduction of 20 w/w % trehalose and looked qualitatively the same. Next, the supported bilayer was imaged after drying and several regions of the dried bilayer are shown in Figure 3.2B. It should be noted that when trehalose was not introduced to the membranes that nearly complete delamination of the membrane was observed. The data in Figure 3.2C were taken after the bilayer was rehydrated with bulk deionized water. As shown in the figure, the images look similar to those taken before the addition of the sugar solution and air exposure. Moreover, FRAP data reveals that the bilayer retains the identical diffusion constant and nearly the identical mobile fraction of lipids (Table 3.1). The procedure described above was repeated with 15 w/w % trehalose. The experiments showed a

Table 3.1. Diffusion constants, mobile fractions, and percent delaminations of lipid bilayers dried and rehydrated in the presence of free sugars.

	control, 10^{-8} cm^2/s	% recovery	dried-rehydrated, 10^{-8} cm^2/s	% recovery	Approx. % delamination
20% Trehalose	3.2±0.4	96±1	3.4±0.5	93±0.8	5
15% Trehalose	3.6±0.7	96±1	3.0±0.6	98±1.0	11
10% Trehalose	4.8±0.7	98±1	4.9±0.3	98±1.2	40
5% Trehalose	5.1±1.0	97±1	4.3±1.0	98±0.4	45
20% Maltose	3.5±0.4	96±2	NM	--	44
15% Maltose	2.9±0.4	95±1	NM	--	11
20% Glucose	3.7±0.8	96±2	SD	--	100
15% Glucose	3.0±0.7	96±1	SD	--	100
20% Sucrose	3.4±0.9	96±1	SD	--	61
15% Sucrose	3.8±0.5	95±1	SD	--	97
20% Lactose	4.6±0.5	96±3	SD	--	95

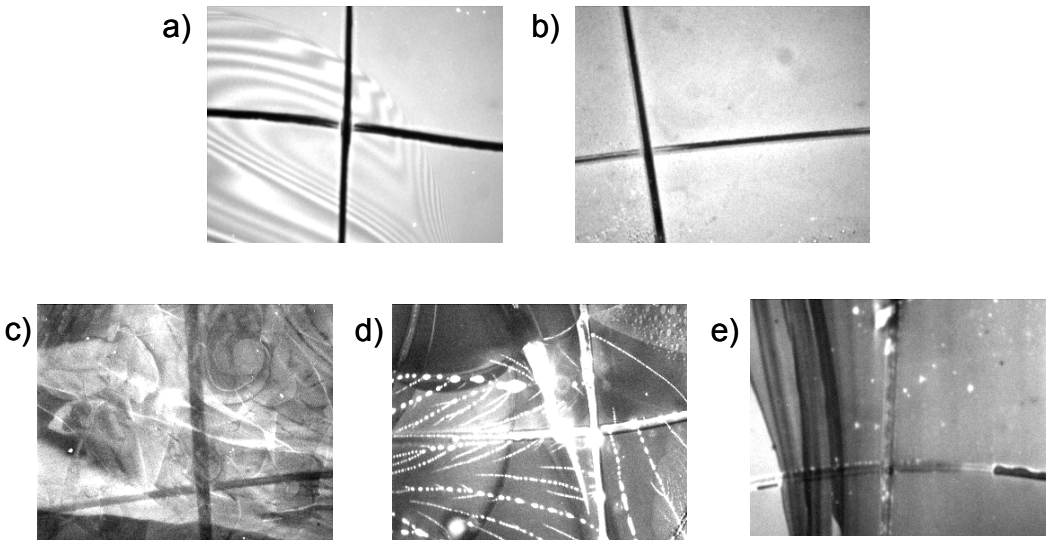

Figure 3.3. Images of dried bilayers exposed to various sugars. a) trehalose, b) maltose, c) glucose, d) sucrose, and e) lactose.

slightly larger area where delamination occurred. On the other hand, the undelaminated areas of the supported membrane were still fully fluid. When 10 and 5 w/w % trehalose solutions were used, fluid bilayers could still be obtained on some portions of the surface, but delamination the bilayer was more frequent at 40 and 45% respectively (Table 3.1).

While experiments involving sufficient trehalose concentration clearly demonstrated that bilayers dried in its presence can be preserved, the remaining sugars in this study were far less effective. For example, when glucose and sucrose were employed, the subsequently rehydrated bilayers exhibited extensive delamination. This was manifested by large dark areas in the fluorescence images (Figure 3.3c-e). Moreover, upon rehydration, FRAP experiments revealed that any bilayer fractions left behind was immobile. Thus, essentially 100% of the surface area showed damage to the bilayer as a result of complete delamination.

The results obtained with maltose were somewhat more intriguing, despite the fact that maltose has had little attention in the area of anhydrobiotic research. As seen in Figure 3.4, it appeared that the bilayer exposed to 20 w/w % maltose experienced no more delamination from the surface than was observed upon the introduction of trehalose (Figure 3.3 a). When FRAP was performed, however, all the regions tested were immobile. In order to determine whether or not all reducing sugars acted like maltose, lactose, another reducing disaccharide, was tested as a membrane preservation agent as well. Unlike maltose, however, lactose provided little protection and resulting rehydrated bilayers were so extensively delaminated that FRAP data could not be taken.

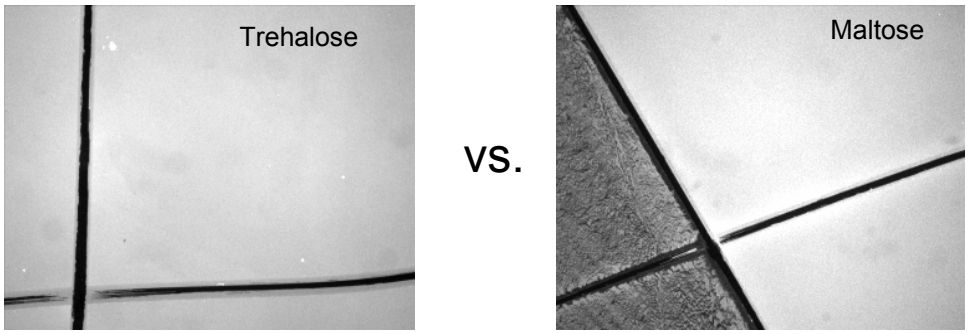

Figure 3.4. Comparison of rehydrated bilayers from trehalose and maltose protection.

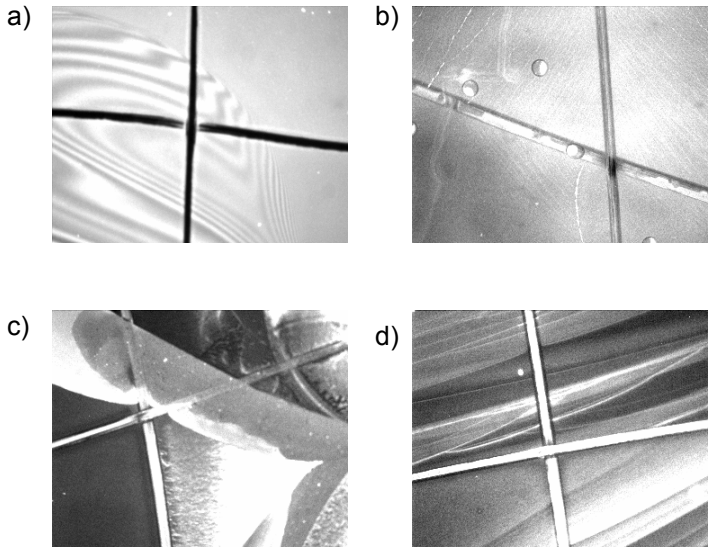

Figure 3.5. Images of dried bilayers exposed to various glycolipids. a) trehalose, b) ganglioside G_{M1}, c) 18:1 lactosyl PE, d) glucosylceramide.

Protection of Supported Lipid Bilayers by Glycolipids. In addition to sugar solutions, the incorporation of glycolipids into the membrane was assayed for their ability to prevent membrane delamination upon air exposure. Three candidate lipids were chosen: G_{M1} ganglioside, lactosyl PE and glucosylcerebroside (Figure 3.1). Vesicles were prepared at concentrations of 10 mol % glycolipid in POPC with 0.1 mol % Texas Red DHPE. The motivation for choosing each glycolipid was straightforward. Lactosyl PE and glucosylcerebroside are lipid-conjugated analogs of glucose and sucrose. Therefore, we were curious as to whether covalently attaching the sugar molecules to the membrane would afford air stability when the free sugars did not. The last species, G_{M1}, was chosen because like polyethylene glycol,[49] the particularly large glycolipid should protect the bilayer against delamination by increasing the bending elastic modulus of the membranes.

FRAP data were obtained before and after rehydration for the three glycolipid systems. As demonstrated in Figure 3.5, the bilayer with G_{M1} showed some protection against delamination while the bilayers in the other two cases were completely destroyed. FRAP data revealed that the G_{M1} containing membrane was completely immobile after air exposure. These results are summarized in Table 3.2.

Discussion

The results from sugar solutions and glycolipids provide new insight into the role of carbohydrates in the preservation of bilayers. Trehalose, studied previously on liposomes and more recently on bilayers,[82] is know to undergo vitrification and form a

Table 3.2. Diffusion constants, mobile fractions, and percent delaminations of dried and rehydrated bilayers containing glycolipids.

	control, 10^{-8} cm^2/s	% recovery	dried-rehydrated, 10^{-8} cm^2/s	% recovery	Approx. % delamination
20% Trehalose	3.2±0.4	96±1	3.4±0.5	93±0.8	5
10% GM1	2.9±1.1	94±2	SD	--	32
10% Lactosyl	3.3±0.5	98±1	SD	--	79
10% Glucosyl	3.3±0.7	97±1	SD	--	62

glassy state with a high glass transition temperature (T_g) atop a bilayer.[64, 72, 86] In my studies, the formation of the sugar glass was demonstrated to protect the bilayer during exposure to air. Biological preservation, however, occurs not just through vitrification, but also in conjunction with the replacement of water by sugar molecules that therby interact with lipid headgroups.[57] When either vitrification or water replacement is absent, the solid supported membrane was not protected. After observing the differing degrees of bilayer delamination experienced when trehalose concentrations were varied, we also believe that at the biological concentration of 20% dry weight,[87] the greatest degree of bilayer protection is acheived.

Disaccharides, such as trehalose, are proposed to provide better protection than monosaccharides due to flexibilities that allow for them to position themselves between lipid headgroups to maximize lipid headgroup contact.[66] This dependence on sterics was seen in the sugar solution studies as bilayers protected with glucose suffered extreme delamination upon rehydration while samples using trehalose exhibited very little. However, protection based on steric dependence would indicate that sucrose would provide comparable protection to that of trehalose. In all samples exposed to sucrose, delaminated areas were prevalent and the level of preservation was very minimal indicating that perhaps sucrose does not lower the T_m of the bilayer as much as trehalose.

The reducing versus non-reducing nature of the sugars was also tested by comparing bilayers treated with trehalose and maltose. Rehydrated bilayers treated with both sugars suffered less than 50% delamination. Since bilayers exposed to maltose do not preserve the supported bilayer structure while those exposed to trehalose do, it is

apparent that these disaccharides interact with the membrane quite differently. Upon further investigation, we believe the contrast in rehydrated bilayers has to do with the different structures of the two sugars in solution. Previous studies of the interactions between trehalose and the lipid bilayer indicate that sugar molecules replace water molecules around the polar headgroups and form hydrogen bonds with the headgroups.[57, 61] Upon examination of crystal and molecular studies of trehalose,[88, 89] it becomes evident that the two monosaccharide units fold together compactly to form a clam-like structure (Figure 3.6). It is our belief that because of its cupped shape, trehalose is able to penetrate deeply into the air/water interface of a bilayer forming a sugar glass and suppressing the T_m so that upon rehydration, delamination is avoided and all bilayer function remains. In the case of maltose, however, crystal structures[90] indicate that the bond angle between the monosaccharide units is much larger than in trehalose thereby creating a flatter sugar structure (Figure 3.6). While maltose atop a lipid bilayer will form a sugar glass that will prevent bilayer delamination, the sugar does not have the structural ability to penetrate deeply enough into the bilayer to sufficiently suppress the T_m. Therefore, upon rehydration, a bilayer exposed to maltose will exhibit minimal defects due to an absence of delamination, but will not diffuse. In the biological sense, a cellular organism's preference to non-reducing sugars may stem from the fact that reducing sugars are often involved in Maillard reactions,[91] leaving non-reducing sugars available for accumulation in anhydrobiotic organisms and subsequent preservation during dehydration.[34] Additionally, recent experimentation has shown that maltose does not depress the T_m of the lipids to the extent that trehalose does

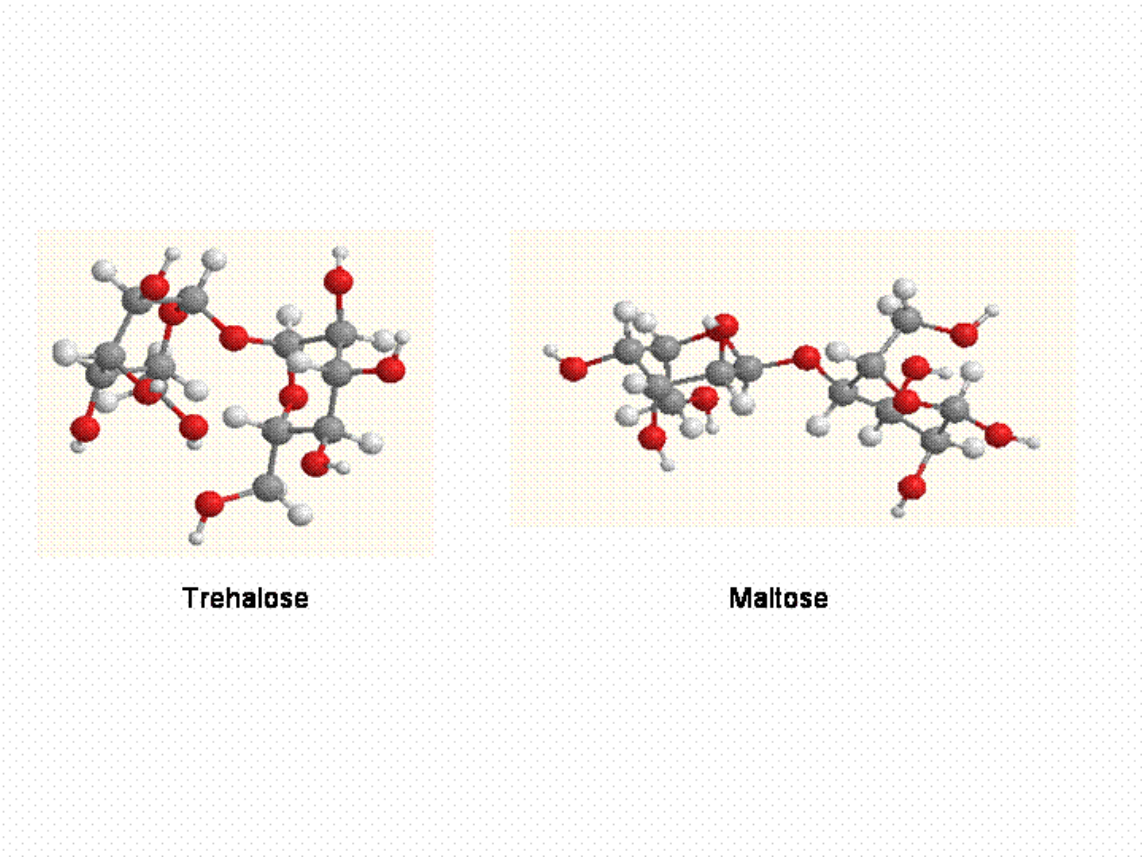

Figure 3.6. 3-D chemical structures of trehalose and maltose.

thereby preventing recovery of full bilayer function.[92] Before being able to establish the ineffectiveness of reducing sugars as protecting agents, experiments were performed where lactose, another reducing disaccharide was tested to see if it had the same effect as maltose. Results of the lactose experiment showed that lactose, unlike maltose, offers no protection to the lipid bilayer.

The results of the glycolipid study provided new insight into the role of sugar/lipid moieties as bilayer preservation agents as well as the presence of a possible size correlation. Of the three glycolipids employed, G_{M1} ganglioside offered the most protection to the bilayer. This is presumably due to its large size, which should inhibit the membranes ability to peel away from the interface due to an increase in the bending elastic modulus. Nonetheless, G_{M1} afforded no protection against membrane delamination whatsoever. Indeed, any lipids remaining on the surface showed no long range fluidity in FRAP experiments. Perhaps the lack of protection offered by GM1 is due to the fact that G_{M1} clusters in the bilayer via hydrogen bonding.[88, 89]

Steric arguments may possibly explain why bilayers containing sugar headgroups protect inadequately when compared to free sugars such as trehalose. One of the primary explanations concerns the geometries of the glycolipids versus the free sugars. While the sugars in the free-sugar study were in bulk solution with freedom of motion, the glycolipids were permanently held to certain lipids within the bilayer. Also, the bulk sugars had the capability of positioning themselves in a three dimensional geometry as sugars could lie across the bilayer as well as atop other sugars. The glycolipids, however, could only interact via their fixed two dimensions thereby limiting the

available sugar protection. Another explanation is that while the concentration of bulk sugar exposed to the bilayer can be as high as 20 w/w % as is the case of trehalose in biological systems, the amount of glycolipids per bilayer mixture is limited to roughly 10 mol %. Studies performed before anhydrobiotic simulations showed that G_{M1} concentrations greater than 10 mol % produce immobile lipid bilayers in standard hydrated conditions which automatically establishes the maximum amount of glycolipids that could be used to model bilayers made in the free-sugar study.

Results from both the free and glycolipid studies were used to create side-by-side comparisons of similar free and glycolipids used. For example, it became possible to look at results for glucose and glucosylcerebroside as well as lactose and lactosyl PE simultaneously to see if there was a correlation between degree of protection and how the sugar interacted with the bilayer. In the case of both sugars and their attached derivatives, it was clear that neither species, free or attached, provided sufficient protection to aid in the preservation of a bilayer. This realization supports studies that have examined the roles of glycocalix components, such as the attached-sugar lipids, or glycolipids. While it is known that the glycocalix contains a variety of different glycolipids,[90] their purpose involves functioning like cell adhesion rather than preservation.[91] The results obtained in this study support this fact, as the three variations of glycolipids used provided no protection to dehydrated bilayer systems and therefore reside in the cellular membrane for different functions.

CHAPTER IV

CONCLUSIONS

The overall goal of the above thesis was to successfully mimic the biological process of anhydrobiosis on supported lipid bilayers. In achieving this, it would be possible to create working biosensors that could be dried in the presence of saccharides and then later rehydrated in order to return to their original state. Essentially, protection could be afforded to the sensor without altering the bilayer in any means. It became clear upon the initiation of this project that not only would the process of anhydrobiosis be extensively examined, but the nature of each of the different sugars used would become a core focus as well.

While mimicking anhydrobiosis has consistently been studied on liposomes, very little research has been performed on supported lipid bilayers, models of the cell membrane. Even though trehalose has long been considered the best sugar for protecting bilayers subjected to dry conditions, other sugars were also studied as bilayer protectants. The free-sugar study of this experiment, however, indicated that when compared to glucose, sucrose, maltose and lactose, trehalose achieves full bilayer recovery after rehydration. Additionally, at the biologically relevant concentration of 20 w/w %, rehydrated bilayers only had approximately 5% delamination, indicative of superior protection.

An anomaly of this study, however, was that the disaccharide maltose visually appeared to protect bilayers, yet when FRAP was attempted, the bilayer was immobile and therefore non-functioning. It has been hypothesized that because maltose and

trehalose exist in different conformations, the two exhibit differing degrees of protection. More specifically, trehalose is formed by a C1α-C1α linkage of its two glucose subunits while maltose contains a C1α-C4α linkage, the more common configuration of disaccharides. Subsequently, the structure of trehalose resembles a "clam-shell" with a bi-dentate tendency and is therefore able to penetrate further into the bilayer thereby preventing delamination, yet allowing full function to return. Current sum frequency generation (SFG) spectroscopy experiments within our laboratory support that due to its compact structure, trehalose tends to partition closer to the hydrophobic tail region of lipid bilayers than maltose does.[92]

The novel aspect explored in experiments was the role of lipids with sugar headgroups and whether or not these entities provided the same protection as the free sugar trehalose. If protection could be obtained with glycolipids, their role in the glycocalix could be more versatile than originally thought. Results, however, indicated that protection with the three glycolipids selected, including one much larger than trehalose, was not achieved primarily due to differing geometric capabilities and concentration coverage on the bilayer. Direct comparisons between similar free sugars and glycolipids further demonstrated the inability of attached sugars to aid in bilayer preservation verifying that glycolipids in the cellular glycocalix are not intended for use as a bilayer protectant.

The studies of free sugars in solution and glycolipids by fluorescence microscopy further demonstrated the superiority of trehalose as a bilayer preservation agent on supported lipid bilayers. Upon thorough investigation of a handful of sugars it becomes

evident that using trehalose on supported bilayer systems creates a system that can not only mimic cellular membrane systems, but can also be employed as a sensor device capable of stability in the absence of water. With the capacity to thrive in even the driest conditions, air-stable SLBs protected with the likes of trehalose show promise for numerous practical and scientific applications.

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